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Short communication

Association between hematological status at weaning and weight gain post-weaning in piglets

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ABSTRACT

The objective of the study was to determine the associations between hematological parameters at weaning and post-weaning weight gain in piglets. Five well-managed conventional herds of average herd size of 1000 sows were selected. All herds provided a single injectable iron (either i.m. or s.c, 200 mg) supplementation from different commerical products at 3–4 days of life. Within each herd, litters belonging to a weekly farrowing batch close to weaning were identified and among them 20 litters were chosen randomly after exclusion of nursing sows. Within each litter, a random piglet was selected. EDTA and non-stabilized blood sample was taken from the anterior Vena cava of each piglet 1–3 days before weaning (average weaning age of 25.7 days) and analyzed by complete hematology including serum iron and total iron binding capacity (TIBC). The associations between each measured hematological parameter and the average daily gain (ADG) of piglets in a three-week period were tested using PROC MIXED procedure in SAS. A total of 99 piglets were included in the study but 3 were lost during follow up. The average hemoglobin concentration of piglets was 121.5 ± 15 g l^{-1} and the ADG was 202.9 ± 78.4 g day^{-1}. A positive association between hemoglobin and ADG in the three-week period ($p=0.0003$) was observed. Also, red blood cells ($p<0.0001$) and hematocrit ($p=0.0005$) had positive association with ADG while red blood cell distribution width ($p=0.05$), hemoglobin distribution width ($p=0.04$), and reticulocyte red cell distribution width ($p=0.01$) had a negative association with ADG. Hence the results indicate that improved hematological status at weaning is positively correlated to weight gain post-weaning. An increase in 10 g hemoglobin/l blood corresponded to a weight gain improvement of 17.2 g daily weight gain in the 3 weeks post-weaning period. The strategies for improving the hematological values of piglets at weaning need to be further investigated.

1. Introduction

Piglets are prone to iron deficiency anemia for several reasons including, very low iron stores at birth, inadequate supply from sow milk, and rapid growth rate (Venn et al., 1947; Rincker et al., 2004). Therefore, it is a standard procedure to iron supplement piglets in the first days of life and in some herds also later during the suckling period. However, it is unknown whether optimal hematological values to promote rapid growth of piglets are achieved under production conditions.

Several studies have focussed on the level of hemoglobin and the cut-off values for anemia in piglets (Furugouri, 1975; Egeli and Framstad, 1998). However, even though hemoglobin (Hb) values above anemia cut-off is reached, there may still be a positive production effect of somewhat higher values. Aukett et al. (1986) found an increased rate of weight gain in children treated with iron compared to untreated children. Gentry et al. (1997) have demonstrated that piglets with greater Hb levels at weaning had greater average daily gain post-weaning. The effect of other hematological parameters on the growth rate of pigs has not been investigated yet.

Some studies have suggested that Hb is not a sensitive indicator of early fall in iron status (Cook, 2005; Svoboda et al., 2008) and several pig and human studies suggest that mean corpuscular volume, mean corpuscular hemoglobin, red cell distribution width, serum iron, total iron binding capacity, transferrin saturation, serum ferritin and reticulocyte parameters are earlier indicators of iron deficiency (Lipschitz et al., 1974; Furugouri et al., 1982; Smith et al., 1984; McClure et al., 1985; Macdougall et al., 1992; Andrews et al., 1994; Ilić et al., 2006; Mast et al., 2008; Svoboda et al., 2008). We hypothesize that Hb and hematological parameters influence the growth rate of piglets and therefore, the aim of this study was to investigate the associations between hematological parameters at weaning and post-weaning weight gain in piglets.

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2. Materials and methods

This study was conducted in accordance with the guidelines of the Danish Ministry of Justice Act No. 382 (June 10, 1987) and Acts 333 (May 19, 1990), 726 (September 9, 1993), and 1016 (December 12, 2001) with respect to animal experimentation and care of animals under study.

Five high-performing conventional farrow-finish sow herds were selected for the study (Table 1). Within each herd, 20 litters ready to weaning within 1–3 days and belonging to the same farrowing batch were selected by convenience sampling. The average weaning age of piglets in each herd is presented in Table 2. In each litter, a random piglet was selected as the sixth clinically healthy individual when counting snouts from the pen side of the observer. In total, 99 piglets were studied. Blood was collected from each selected piglet and subjected to complete hematological analysis including serum iron. The procedures of herd selection, sow and piglet selection and method of blood sampling have previously been described in details in Bhattarai and Nielsen (2015). The present study constitutes a follow-up study for a fraction of the piglets described in Bhattarai and Nielsen (2015).

The following recordings were made within each litter: Farrowing date, litter size as counted at the day of visit, sex of the selected piglet, and parity of the sow. The selected piglets were weighed individually and examined for obvious signs of anemia (pale skin) or clinical signs of disease at the time of blood collection. The piglets were ear tagged with individual identification numbers for follow up.

Three weeks after weaning, body weight of all the piglets was recorded again. The average daily gain (ADG) was calculated as the difference between the weaning weight and the 3-week post-weaning weight, divided by the number of days between weighing.

2.1. Hematology

The EDTA stabilized blood samples were analyzed for hematological indices; erythrocyte count (RBC), leucocyte count (total leucocyte count and differential leucocyte count), platelets, mean platelet volume (MPV), red blood cell distribution width (RDW), hemoglobin concentration (Hb), hemoglobin distribution width (HDW), hematocrit (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC). Reticulocyte indices were also analyzed which included reticulocyte count (absolute and relative), reticulocyte hemoglobin content (Chr), mean reticulocyte corpuscular hemoglobin concentration (CHCMr), reticulocyte cellular volume (MCVr), reticulocyte red cell distribution width (RDWr), and reticulocyte hemoglobin distribution width (HDWr). The serum samples were analyzed for serum iron and total iron binding capacity (TIBC). Transferrin saturation (TfS) was calculated using the formula: TfS (%) = Serum iron / TIBC $\times$ 100.

Complete hematology was tested using Advia 2120i Hematology System (Siemens healthcare diagnostics Inc. Tarrytown, NY 10591, United States) while serum iron and TIBC were tested using Advia 1800 Chemistry System (Siemens healthcare diagnostics Inc. Tarrytown, NY 10591, United States) at the Central laboratory, Department of veterinary clinical and animal sciences, University of Copenhagen. Method validations were according to protocols of the manufacturer (Siemens healthcare diagnostics, 2008).

Table 1
Descriptive data of the herds that participated in the study.

	Herd 1	Herd 2	Herd 3	Herd 4	Herd 5
Herd size	1101	1100	1155	1201	940
Weaning age (day)	26.3	24.2	27.8	24.8	25.7
Total born alive/litter (piglets)	15.7	15.6	15.7	14.5	14.3
Age at iron injection (day)	3–4	3–4	4	4	3
Iron brand name[1]	Solofer[a]	Ursoferran[b]	Ursoferran[b]	Hyofer[c]	Hyofer[c]
Dose/Route of iron[2]	1 ml/i.m.	1.1 ml/i.m.	1 ml/i.m.	1 ml/s.c.	1.5 ml/i.m.
Creep feed start	5 days	7 days	10 days	6 days	2 wks

[1] Each injectable iron product contains 200 mg iron dextran per ml.
[2] i.m. = Intramuscular; s.c. = Subcutaneous.
[a] Pharmacosmos A/S, Holbæk, Denmark.
[b] Serumwerk Bernburg AG, Bernburg, Germany.
[c] Salfarm Danmark A/S, Kolding, Denmark.

Table 2
Description of piglet growth performance by herd[a].

Herd	No. of piglets studied	Age at weaning (days)	Weight at weaning (kg)	Weight post-weaning (kg)	ADG[b] (g/day)
1	20	26.2 ± 0.6	6.9 ± 1.2	11.3 ± 1.9	209.3 ± 56.2^{a}
2	18	24.2 ± 1.5	6.0 ± 1.8	10.4 ± 2.8	210 ± 66.0^{a}
3	17	27.8 ± 3.4	6.1 ± 1.2	11.1 ± 2.5	232.7 ± 79.4^{a}
4	19	24.8 ± 1.5	5.4 ± 1.0	10.6 ± 2.5	245.5 ± 86.1^{a}
5	19	25.7 ± 0.8	6.5 ± 1.1	9.4 ± 1.4	118.8 ± 31.0^{b}
Mean		25.7 ± 2.2	6.2 ± 1.3	10.6 ± 2.3	202.9 ± 78.4

*Different superscripts within ADG column indicate significant difference ($p < 0.05$).
[a] Values are presented as mean ± standard deviations.
[b] ADG = Average daily gain. All the piglets were weighed 3 weeks after weaning.

2.2. Statistical analysis

Data analysis was performed using SAS (SAS 9.3, SAS institute Inc, Cary, North Carolina). The associations of each of the measured hematological parameters on the ADG of piglets were tested individually using linear mixed model with PROC MIXED procedure in separate models and the estimates were calculated using SOLUTION option. Farm was considered as a random variable during each analysis with weight at weaning, sex, parity of sow, and litter size as other explanatory variables. The difference in ADG among farms was determined using general linear model with PROC GLM. Pearson's correlation coefficients between hematological parameters were calculated using PROC CORR procedure. Statistical significance was set at $p < 0.05$ for all the tests.

3. Results

Altogether 99 piglets were included in the study. Serum sample was missing from six piglets. The results from 15 EDTA blood samples were not included in the analysis (EDTA blood either not taken/analyzed or clotted). Three piglets were lost during follow up. The average daily gain of the piglets was 202.9 ± 78.4 g day^{-1} (Table 2) and the mean Hb concentration was 121.5 ± 15 g l^{-1} (Table 3). The mean values of selected hematological parameters (those showing association with growth rates) are shown in Table 3. Correlations among Hb, RBC, HCT, RDW, HDW and RDWr are only reported as these parameters showed association with growth rates (Table 5).

The associations between individual hematological parameters and ADG of piglets are presented in Table 4. Only statistically significant parameters are presented. ADG post-weaning had a significant association with Hb (Figs. 1 and 2), RBC, HCT, RDW,

Table 3
Hematological values of piglets at weaning[a].

Parameter[b]	*N*	Mean ± SD	Minimum	Maximum
Hb, g/l	84	121.5 ± 15	74.1	151.4
RBC, 10^{12}cells/l	85	6.3 ± 0.6	4.4	7.9
HCT, l/l	85	0.3 ± 0.04	0.2	0.4
RDW, %	85	18.2 ± 2.9	14.5	32.8
HDW, mmol/l	85	1.3 ± 0.1	1.1	1.7
RDWr, %	85	15.4 ± 2.6	13.1	32.4

[a] Parameters that showed significant associations with ADG are presented.
[b] Hb = Hemogobin; RBC = Red blood cell count; HCT = Hematocrit; RDW = Red blood cell distribution width; HDW = Hemoglobin distribution width; RDWr = Reticulocyte red cell distribution width.

Table 4
Associations between hematological parameters at weaning and ADG (g) three weeks post-weaning.[a]

Parameter[b]	Estimate	SE	*P*-value
Hb			
Intercept	−155.66	72.59	0.09
Hb, g/l	1.72	0.45	0.0003
Weaning wt., kg	23.89	5.18	< 0.0001
RBC			
Intercept	−182.29	68.64	0.05
RBC, 10^{12} cells/l	38.43	8.83	< 0.0001
Weaning wt., kg	22.61	4.50	< 0.0001
HCT			
Intercept	−149.48	72.37	0.10
HCT, l/l	539.36	147.52	0.0005
Weaning wt., kg	23.35	4.61	< 0.0001
RDW			
Intercept	152.33	62.06	0.07
RDW, %	−4.83	2.45	0.05
Weaning wt., kg	22.25	4.89	< 0.0001
HDW			
Intercept	254.65	102.17	0.06
HDW, mmol/l	−144.24	69.77	0.04
Weaning wt, kg	22.68	4.88	< 0.0001
RDWr			
Intercept	153.81	56.09	0.05
RDWr, %	−6.29	2.57	0.01
Weaning wt., kg	23.48	4.85	< 0.0001

[a] The results presented are the outcomes from individual models run separately for each hematological parameter with explanatory variables. Only significant parameters are presented.
[b] Hb = Hemoglobin, RBC = Red blood cell count, HCT = Hematocrit, RDW = Red blood cell distribution width, HDW = Hemoglobin distribution width, RDWr = Reticulocyte red cell distribution width.

Table 5
Pearson Correlation Coefficients among hematological parameters of piglets at weaning[a].

Parameters[b]	Hb	RBC	HCT	RDW	HDW
Hb, g/l					
RBC, 10^{12}cells/l	0.63*	–			
HCT, l/l	0.94*	0.68*	–		
RDW, %	−0.56*	−0.04‡	−0.44*	–	
HDW, mmol/l	−0.44*	−0.07‡	−0.49*	0.62*	–
RDWr, %	−0.60*	−0.20‡	−0.58*	0.74*	0.63*

* Significant at < 0.05.
‡ Non-significant.
[a] Parameters that showed significant associations with ADG are only presented.
[b] Hb = Hemoglobin, RBC = Red blood cell count, HCT = Hematocrit, RDW = Red blood cell distribution width, HDW = Hemoglobin distribution width, RDWr = Reticulocyte red cell distribution width.

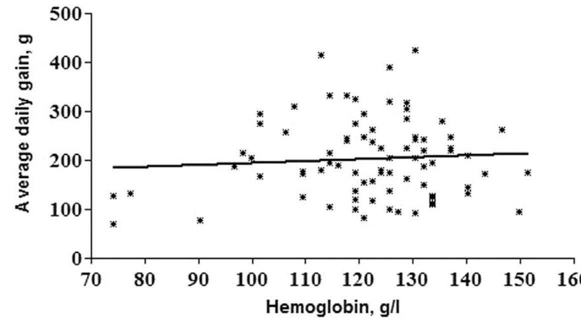

Fig. 1. Relationship between hemoglobin concentration at weaning and average daily gain (ADG) three weeks post-weaning in piglets (Raw data). Pearson correlation coefficient (r) = 0.07 (P = 0.49).

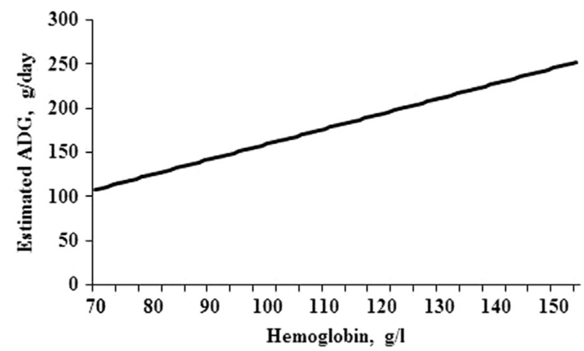

Fig. 2. Association between hemoglobin concentration at weaning and average daily gain (ADG) three weeks post-weaning in piglets based on the final model. ADG was calculated with 6 kg of weaning wt.

HDW, and RDWr. ADG was significantly associated to weaning weight of piglets in each of the analysis while, litter size, parity of the sow, and sex of the piglet were not associated to ADG in any of the analysis. The results indicate that an increase in 10 g hemoglobin/l blood corresponded to a weight gain improvement of 17.2 g (Table 4).

4. Discussion

This study indicates that the improved hematological status at weaning was associated with average daily weight gain in piglets post-weaning. We have found previously (Bhattarai and Nielsen, 2015) that several indices of immature erythrocytes, serum iron, and TIBC are earlier indicators of recent erythropoetic activity in weaning piglets than indices of mature erythrocytes. However, in the current study we did not see an association between those early indicators of iron deficiency and growth except with reticulocyte distribution width. It seems that the body weight of piglet is not affected until the iron status is too low to affect the indices of mature erythrocytes such as Hb and RBC.

It has been shown previously that an additional iron injection on day 21 resulted in a greater growth post-weaning in piglets (Kamphues et al., 1992). In a Danish study, an additional 200 mg iron injection at the age of 20 days in piglets resulted in a significantly greater growth rate of 20 g per day for the first 15 days after weaning compared to the piglets receiving only one injection at 3 days of age (Haugegaard et al., 2008). This may be due to improved oxygen transport, immune function (Schrama et al., 1997), vitality, metabolism (Gentry et al., 1997) and intestinal health (Larkin and Hannan, 1984) in the piglets as hemoglobin levels increase. Hansen et al. (2010) indicated that during the first weeks after weaning, the regulation of intestinal absorption may not be fully functional as the mRNA levels for the iron transporters divalent metal transporter 1 and ferroportin are not upregulated in the duodenal mucosa of piglets until between 36 and 47 days of age. This could probably partly explain the importance of improved hematological status at weaning for the improved post-weaning growth in piglet.

There are other studies which did not find effect of Hb or iron status on growth rate of piglets (Murphy et al., 1997; Bruininx et al., 2000). Amine et al. (1972) demonstrated that body weight is only slightly affected in extreme anemia in rats but the scenario could be different for pigs. Recently, Jolliff and Mahan (2011) have demonstrated that the piglets injected with 200 mg iron at birth with an additional 100 mg at 10 days had greater Hb and hematocrit through 14 days post-weaning and greater HCT values through 21 day post-weaning than in piglets injected with single 200 mg iron, however there was no effect of these values on growth at weaning or later throughout the post-weaning period of 21 days.

Our results have shown that there is a significant effect of weaning weight on post-weaning weight gain in piglets which was previously confirmed by other studies (McConnell et al., 1987; Tokach et al., 1992; Dunshea et al., 2003).

The results of the study provide an indication that Hb, RBC, HCT, RDW, HDW, and RDWr are related as they have a similar correlation with growth rate in piglets. However, Hb measurement alone is a routine diagnostic method for the determination of iron adequacy in swine herds. Furthermore, investigations on on-farm device such as Hemocue® which conveniently measures Hb concentration in piglets are being carried out (Maes et al., 2011; Kutter et al., 2012) and these types of devices do not allow measurement of other hematological parameters. Therefore Hb alone may be used as a predictor of growth rate in piglets in herd health management programs.

We had expected that growth in piglets might be associated to leucocyte and platelet values as studies suggest that these parameters are related to iron deficiency anemia in human patients (Buyukavci et al., 2010; Habis et al., 2010; Özcan et al., 2011). Clinical and *in vitro* studies also suggest that thrombocytosis occurs during iron deficiency anemia (Buyukavci et al., 2010). The number of granulocytes is higher in patients with iron deficiency compared to healthy subjects but the total leucocyte count remains unaffected. Despite this, we did not find any association between these parameters and growth in piglets.

It is well established in human studies that other hematological parameters in addition to hemoglobin change during iron deficiency and anemia (McClure et al., 1985; Macdougall et al., 1992; Mast et al., 2008). However, the exact timing of change and the effect of iron in all these parameters is still unknown. Therefore, it is difficult to interpret these parameters in relation to growth rate in piglets. The average Hb values of piglets were above the reference range reported in literature which lies between 90–112 g/l (Thorn, 2010). However, it is unknown if these values allow for the maximum growth of modern piglets. The recommended optimum values for other hematological and hematochemical values have not been set for pigs, though reference intervals for some parameters can be found. Therefore it is difficult to conclude whether all the desired hematological values were met in these piglets.

None of the chosen piglets in our study had obvious signs of anemia. Despite this fact, we found a positive effect of higher Hb values at weaning on weight gain post-weaning. This suggests that even though the recommended Hb values are reached there is still an effect of higher values on growth rate.

Hemoglobin and other hematological parameters in piglets are influenced by iron supplementation. It seems that the iron status of suckling piglets needs to be routinely examined and necessary interventions should be carried out to adjust iron supplementation program in order to achieve high hemoglobin levels.

In summary, our study has shown that the improved hematological status, especially the indices of mature erythrocytes at weaning is positively associated with growth rate of piglets post-weaning. Therefore, the strategies for improving the hematological status of weaning piglets need to be further investigated.

Conflict of interest statement

None

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